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SPECTRAL SENSITIVITIES OF THE EYES OF THE ORB WEB SPIDER *ARGIOPE ARGENTATA* (FABRICIUS)

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ABSTRACT

Spectral sensitivity curves to light between 425 and 650 nm for the four eyes of the spider *Argiope argentata* were determined. Maximum sensitivity was observed at 530 nm for the secondary eyes and at 525 nm for the principal ones. Chromatic adaptation did not affect this maximum, which suggests that there is only one receptor and photopigment in this spectral region.

INTRODUCTION

Recordings from the anterior median eyes of jumping spiders demonstrated the existence of different photoreceptor types, thus confirming earlier behavioral data suggestive of color vision in these animals (Peckham and Peckham 1887, Crane 1949, Kästner 1950, Young and Wanless 1967). According to DeVoe's (1975) intracellular recordings, color vision in the principal eyes of the jumping spider *Phidippus regius* (C. L. Koch) is mediated by three photoreceptor types: UV cells with peak sensitivity at 370 nm; green cells with peak at 532 nm, and UV-green cells, peaking at 370 and 532 nm. In the secondary eyes of this spider DeVoe found only green cells. In the jumping spider *Menemerus confusus* (Bösenberg and Strand), Yamashita and Tateda (1976) found, in addition to UV and green receptors, a blue receptor at 490 nm and a long-wave-length receptor at 580 nm. Their data were based both on intracellular recordings and on chromatic adaptation effects on the electroretinogram (ERG).

Other families of spiders did not exhibit the same color vision abilities as found in jumping spiders. Young and Wanless (1967) conducted a T-maze preference study in seven spider families, and found that only Salticidae exhibited behaviors suggestive of color vision. In ERGs from Lycosidae, DeVoe, Small and Zvargulis (1969) found only one photopigment (green) in the secondary eyes. The principal eyes contained variable amounts of this pigment in addition to an UV absorbing one.

Color vision was only recently studied in spiders of the Araneidae family. The anterior median eyes of *Argiope bruenchini* (Scopoli) and *Argiope amoena* (L. Koch) were found to have three photoreceptors, with maxima at about 360 nm, 480-500 nm and 540 nm (Yamashita and Tateda 1978, 1981). No recordings were reported for the secondary eyes.

The present study, which was started before the publication of Yamashita and Tateda's reports (Tiedemann 1975), brings information about the color vision system of *Argiope argentata* (Fabricius), an orb web spider whose behavior has been extensively studied (Robinson 1969, Robinson and Olazarri 1971, Ades 1973). *Argiope argentata* lives in sunny areas and is diurnal, but it is capable of hunting and of building its web in the dark. Its characteristic thermoregulatory posture (Robinson and Robinson 1978) was shown to be controlled by light rather than by heat (Ades and Kanner 1978). Its choice of the side of the web on which to stand is also light dependent (Ades and Kanner 1979). It can rely on visual stimuli when placing the first threads of the web, as do other orbweavers (Tilquin 1942). There is no doubt that visual cues are of great relevance to several aspects of behavior of this species. There is not, however, any information regarding its color vision abilities. In this report we describe the ERG measured spectral sensitivities of the dark-adapted eyes of *Argiope argentata,* and the results obtained under chromatic adaptation, showing that it has only one receptor type in the spectral range from 425 to 625 nm.

METHOD

Preparation.—The animal was lightly anesthetized with CO_2 and fixed to a black cardboard with adhesive tape over the legs. The cephalothorax was immobilized with acrylic (Simplex dental acrylic). The recording electrode was a cotton wick held in a glass tube pulled out at one end to a capillary tip and filled with insect physiological solution. Contact with the recording system was done through a nonpolarizable Ag-AgCl wire. The indifferent electrode was a Microtode 415 (Transidyne General Corporation) inserted in a leg of the first or second pair just below the surface. Such a preparation could last for at least 12 hours and longer than a week, if the spider received water by means of a capillary tube. The temperature was maintained at 22 to 24°C during the recordings. ERGs were measured with a Tektronix 122 AC amplifier with a 1 s time constant and 50 Hz filter, and a Tektronix 5103/D13 dual beam storage oscilloscope and photographed with a Grass C-4 camera.

The preparation was placed in a light tight box inside a recording chamber. The optical equipment consisted of a double Maxwellian view system with a tungsten halide quartz lamp (Osram 58.8105 100W) as the light source, which permitted focussing through a light pipe (Fiber Lite) of a 3 mm diameter light spot on the eyes. Intensity was controlled by means of glass-mounted Kodak Wratten 96 neutral density filters and duration of the stimulus flash through a photographic shutter (Wollensack). Monochromatic light was filtered out through an interference color wedge (Veril S-200-Leitz), corrected with neutral density filters Kodak-Wratten 96 for equal light energy for wavelengths between 425 nm and 675 nm in steps of 25 nm. The light passed through a 3 mm slit yielding a halfband width of ca. 8.75 nm. A second light source, for the adapting light was a Fiber Lite Illuminator Mod. 172 with a simple Maxwellian system, perpendicular to the first. In the experiments with ultraviolet light, an incandescent UV-lamp, which emitted light between 320 nm and 440 nm with a maximum output at 380 nm (GE Purple X - 250W) was used in connection with an UV filter with a maximum transmittance at 380 nm and an edge at 400 nm (Kodak 18 A), directly on the preparation.

The stimulating light produced at the end of the light pipe a light intensity of (4.24) . 10^{-6}W at 500 nm, so that a 20 ms flash corresponded to about (2.76) . 10^{11} photons. The adapting light yielded, with a red filter with transmittance from 570 nm to infrared (Kodak 23 A), an intensity of (3.60) . 10^{-5}W and with a narrow band blue filter with maximum output at 430 nm (Kodak 48 B), (1.60) . 10^{-5}W. Calibrations of ND filters at each of the wavelengths used were made with a Tektronix radiometer J 16 with J 6502 probe and a Zeiss DMR-21 spectrophotometer.

Procedure.—All recordings were done during the day, between 8 a.m. and 5 p.m. The preparation was dark adapted for at least 30 minutes prior to each experimental session.

For each eye (anterior median, posterior median, anterior lateral and posterior lateral eye) the energy-response functions were determined under conditions of dark adaptation and red-, blue- and white-light adaptations for each of the calibrated monochromatic lights between 425 and 675 nm. Data collected under light-adapted conditions were preceded by 10 min of exposure to the adapting light before measurements were begun. Energy-response functions were obtained at each of these adapting conditions by measurements of ERG amplitude for stimuli of the following mean attenuations: 0; 0.21; 0.53; 0.95; 1.26; 1.60; and 2.13 log. At each attenuation value the spectrum was scanned twice, at 25 nm steps, in opposite directions, with 20 ms long flashes presented at 30 s intervals.

A standard of 550 nm was used throughout the experiment for frequent checks of the stability of the preparation. All data reported are from preparations which remained quite stable, within 2% variation.

RESULTS AND DISCUSSION

Waveform of the ERGs.—The waveform of the ERGs recorded in *A. argentata* is similar for all four eyes and does not differ as a function of wavelength of the test flash, as can be observed in Figure 1. Changes in waveform were only noticed in connection with variation in light intensity. Recordings shown in Figure 2 exemplify such waveform changes. At high intensities the waveform was typically a single rapid negative wave followed by a slow uniform decay. The small positive wave is an artifact of capacity coupling of the AC recording amplifier. At low intensities the ERG consisted of a double negative wave in all eyes except the principal. In the latter, peak latency was intermediate between the two negative waves found for the other eyes.

The shape of the ERG was also examined under chromatic light adaptation, because it has been reported to change in species that have different photoreceptor types, such as the jumping spider, *M. confusus* (Yamashita and Tateda 1976). In the eyes of *Argiope argentata* no change in waveform was observed as a function of wavelength of chromatic adaptation. This was a first indication of the absence of additional photopigments in the tested part of the spectrum (400-600 nm) in the species.

Spectral Sensitivity.—To determine the spectral sensitivity curves of the four eyes, the ERG amplitudes in response to monochromatic lights presented to the dark-adapted eye at 25 nm steps were measured as a function of light intensity over a 2.0 log unit range. The energy-response functions thereby obtained are plotted in Figure 3. The functions are closely parallel for each of the eyes, but

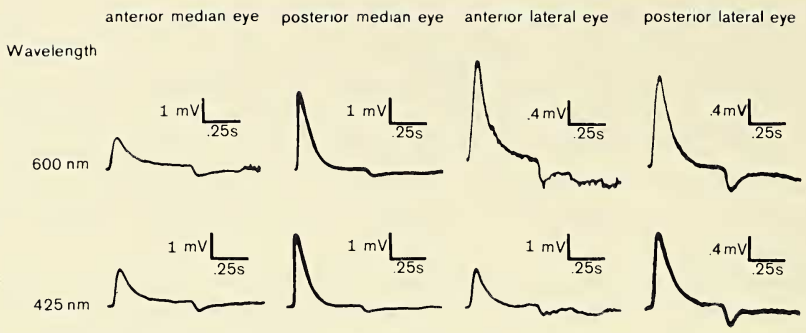

Fig. 1.—ERGs in response to stimuli of 425 nm and 600 nm obtained in the four eyes. Test flash duration was about 1s.

differ in slope from one eye to the other. The posterior median eyes exhibit the shallowest curves, whereas the posterior lateral eyes the steepest ones. The differences could be attributed either to different densities of photoreceptors, or, as suggested by Dahl (pers. comm.), who found similar differences in the eyes of *Aphonopelma,* to the presence of the tapetum in the eyes that exhibited the lesser slope.

To draw the spectral sensitivity curves, the amplitude data collected above were transformed according to the procedure described by Autrum and von Zwehl (1964). This consisted of plotting the mean energy function for each eye and finding the intensity that would have been necessary to elicit a given ERG amplitude. The relative sensitivity is the reciprocal of that intensity.

The entire procedure was repeated under chromatic adaptation to blue and red lights with the purpose of determining whether any of the eyes contained more than one type of visual cell or pigment in the visible part of the spectrum. If this were so, the spectral sensitivity curve obtained under selective chromatic adaptation would be displaced relative to that obtained in the dark adapted state (Wald 1968, Yamashita and Tateda 1976).

Figure 4 shows the spectral sensitivity curves for the four eyes, under dark adaptation and the three light adapting conditions. As can be seen, chromatic adaptation did not change the shape of the spectral sensitivity curves for any of the four eyes. The smooth lines are Dartnall nomogram curves (Dartnall 1953). For the lateral and posterior eyes, the best fitting was a 530 nm nomogram curve, whereas for the anterior median eye a 525 nm nomogram curve yielded the best fit. This discrepancy may be due not to a different photopigment but to differences in the optic media between principal and secondary eyes (Young and Wanless 1967). In the short-wave range of the spectrum the fit of the Dartnall

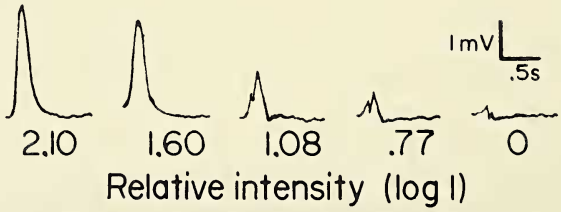

Fig. 2.—ERGs from light adapted posterior lateral eye for 550 nm, light stimuli of five different intensities. Test flash duration was 20 ms.

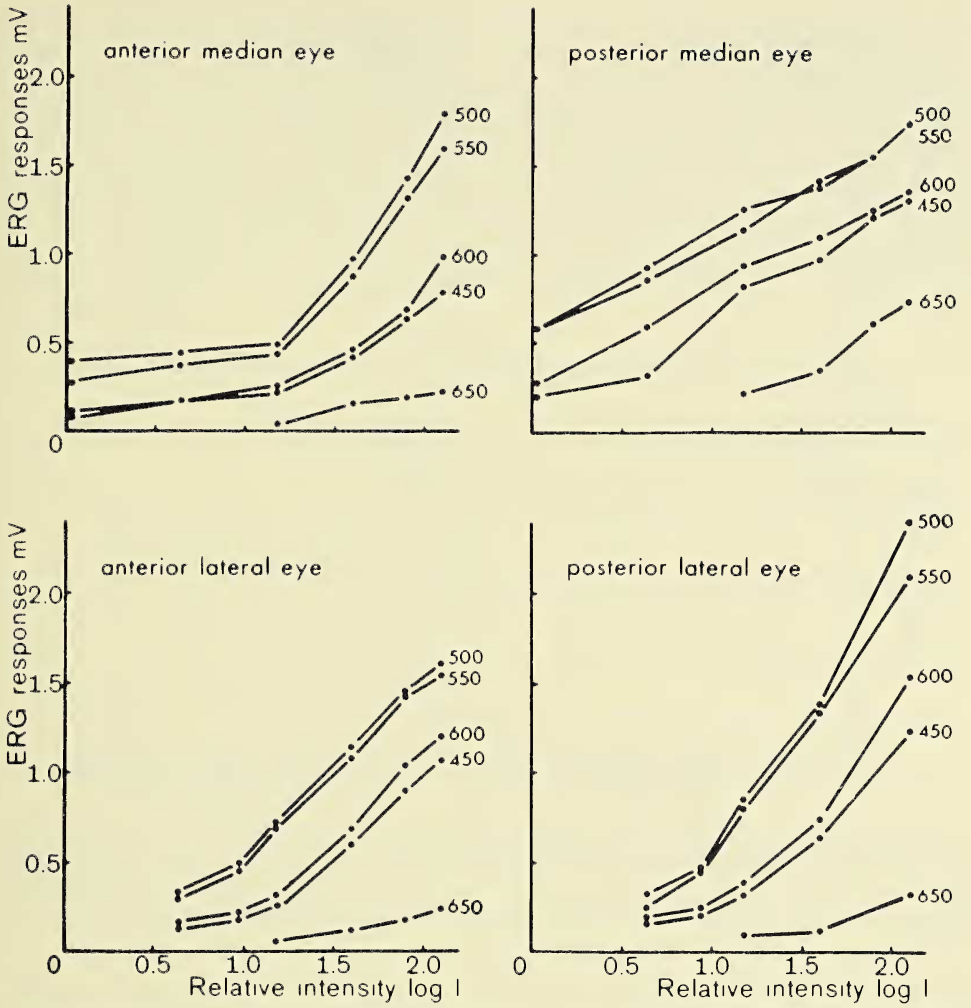

Fig. 3.—Energy-response functions for dark adapted eyes obtained for five different wavelengths (data from one preparation).

nomogram curves was not so good. The sensitivity was less than predicted by the nomogram for all four eyes. The same type of result was found by DeVoe, Small and Zvargulis (1969). This is not, however, the common finding in other arthropods, the rule being that sensitivity in the near UV is higher than predicted by the nomogram curves, as found in the bee (Autrum and von Zwehl 1964), dragonfly (Autrum and Kolb 1968), *Calliphora* and *Periplaneta* (Walther and Dodt 1959), *Musca domestica* (Eckert 1971), *Atta sexdens* (Martinoya, Bloch, Ventura, and Puglia 1975) and several species of crustacea (Scott and Mote 1974). UV sensitivity is nevertheless present in *A. argentata,* as revealed by tests made with UV stimulation (GE Purple X lamp plus Kodak 18 A filter). These tests showed that responses in the UV are present in all four eyes, but are higher in the anterior median eyes than in the other ones. This is in agreement with DeVoe's observations (DeVoe, Small and Zvargulis 1969, DeVoe 1972, 1975), who also found higher sensitivity in the anterior median eyes.

The reason why we did not find evidence for more than one visual pigment in the anterior median eyes of *Argiope argentata,* as found for the Japanese

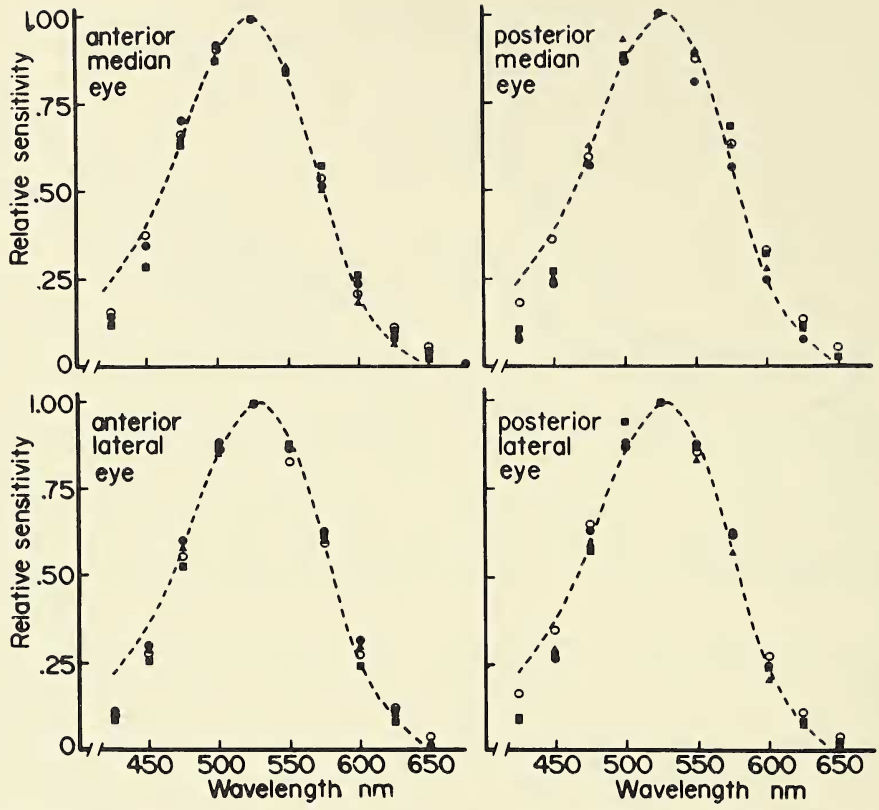

Fig. 4.—Spectral sensitivities of dark (open circle), white (solid circle), blue (square) and red (triangle) adapted eyes (sensitivity expressed as $I/I\lambda$, where I is the intensity needed for a standard response at peak wavelength, and $I\lambda$, the intensity needed for the same response at wavelength λ). The line drawn in each graph is a Dartnall nomogram for a photopigment with λmax at 530 nm in secondary eyes, and 525 nm for the principal eyes.

species, is not clear. This failure to isolate a blue receptor (their "green" cell with peak at 540 nm closely overlaps our data, which peaks at 530 nm) could be attributed either to species differences, or to a lower sensitivity of our technique. It could have also been due to the time of day in which the data were collected. The blue cells found by Yamashita and Tateda were recorded at night. Our data were always collected during the day. Alternatively, Yamashita and Tateda's claim for the existence of a "blue" receptor cannot be regarded as unquestionable. There are two reasons for this. First, the fact that their claim is based on recordings from only two cells. Second, the fact that the peak of the "blue" cell (480-500 nm) falls very close to that of the "green" one. In fact, out of the 24 cells studied by them, only nine had a single peak (three were "UV" cells, two were "blue" cells, and four, "green" ones). It is possible that the spectral sensitivities obtained for the cells classified as "blue" represent the result of electrical coupling between "UV" and "green" ones. A recent article by Horridge, Marcelja, Jahnke and Matic (1983) shows interactions caused by coupling which produced nine different types of spectral sensitivity curves in the butterfly retina. Because coupling appears "to be the rule rather than the exception in visual systems" (Shaw 1981), the identification of spectral sensitivity curves with single pigments or single photoreceptors cannot be made without the meeting of a

number of criteria, as was done by Horridge et al. (1983) for their primary cell types.

Alternatively, more recent work from our group (Souza, Menzel, and Ventura 1985) shows that the flash method of spectral sensitivity measurement yields unreliable data, due to both the lengthy recording sessions, with unavoidable baseline fluctuations, and to the changes in the state of adaptation of the cell with the use of different spectral filters. A much more reliable method called "voltage clamp" by Franceschini (1984) or "constant response" (Hertel and Ventura, in press) avoids both problems and produces reproducible pigment — like spectral sensitivity curves.

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